UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/920,332	08/02/2001	Shailaja Kasibhatla	1735.0470001/RWE/ALS	5774

26111 7590 08/12/2003

STERNE, KESSLER, GOLDSTEIN & FOX PLLC
1100 NEW YORK AVENUE, N.W.
WASHINGTON, DC 20005

EXAMINER
HUYNH, PHUONG N

ART UNIT	PAPER NUMBER
1644	10

DATE MAILED: 08/12/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/920,332	KASIBHATLA ET AL.	
	Examiner	Art Unit	
	Phuong Huynh	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *Three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 June 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-35* is/are pending in the application.

4a) Of the above claim(s) *32-35* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *6&7* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. Claims 1-35 are pending.

2. Upon reconsideration, Group II (Claims 14-27) has been rejoined with Group I.

3. Applicant's election with traverse of Group I, Claims 1-13 and 28-31 drawn to a method of identifying immunosuppressive agents affecting the caspase cascade, filed 6/2/03, is acknowledged. The traversal is on the grounds that (1) the Examiner has not full satisfied the second criteria of MPEP § 803. As such, a search of one group of claims is likely to encompass subject matter pertinent to the patentability of all groups since Groups I and II have been classified in the same class and subclass. Applicant's arguments with respect to rejoining of Groups I and II are moot in view of the rejoining of Groups I and II. Therefore, the requirement of Group I (claims 1-31) and Groups III-IV is still deemed proper and is therefore made FINAL.

4. Claims 32-35 are withdrawn from further consideration by the examiner, 37 C.F.R. 1.142(b) as being drawn to non-elected inventions.

5. Claims 1-31 drawn to a method for identifying an immunosuppressive agent affecting the caspase cascade are being acted upon in this Office Action.

6. Applicant should amend the first line of the specification to reflect the relationship between the instant application and provisional application 60/222,897 filed August 3, 2000 stated on the oath.

7. The international reports and copending USSNs crossed out on PTO 1449 filed 5/10/02 and 8/28/02 have been considered but cross out because said international reports and copending USSNs are not appropriate for an IDS.

8. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claims 1-31 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling only for (1) a method for identifying an immunosuppressive agent comprising the steps of (a) exposing activated or resting T lymphocytes to a test compound dissolved in a solvent or solvent alone (control) for a predetermined period of time at a predetermined temperature; (b) adding a reporter compound wherein the reporter compound is N-(Ac-DEVD)-N'ethoxycarbonyl-R110 of SEQ ID NO: 1, (c) measuring the relative fluorescent intensity of said reporter compound in T lymphocytes exposed to the test compound and T lymphocytes exposed to the solvent alone, (d) calculating the relative potency of said test compound by obtaining the ratio of fluorescent intensity measured for the test compound and the fluorescent intensity measured for the solvent wherein when the ratio is greater than one, the said compound kills active T cells and is a potential immunosuppressive agent, and (2) a method for assaying the potency of a test compound to synergize with a known immunosuppressant by functioning as an activator of the caspase cascade, said method comprises the steps of (a) exposing activated T cell having intact cell membrane to a test compound in the present or absence of a known immunosuppressant for a first predetermined period of time and a first predetermined temperature, (b) adding a reporter compound wherein the reporter compound is N-(Ac-DEVD)-N'ethoxycarbonyl-R110 of SEQ ID NO: 1, (c) incubating the resulting mixture for second predetermined time period at a second predetermined temperature, (d) measuring the relative fluorescent intensity of said reporter compound in T lymphocytes exposed to the test compound in combination with the known immunosuppresant and T lymphocytes exposed to the solvent alone, (e) calculating the relative potency of said test compound by obtaining the ratio of fluorescent intensity measured for the test compound in the present or absence of immunosuppressive agent to determine whether said test compound synergies with said known immunosuppresant as an activator of the caspase cascade, **does not** reasonably provide enablement for any methods for identifying any immunosuppressive agent as set forth in claims 1-31 using any reporter compound having any measurable property which is responsive to any caspase cascade. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in **scope** with these claims.

Factors to be considered in determining whether undue experimentation is required to practice the claimed invention are summarized *In re Wands* (858 F2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). The factors most relevant to this rejection are the scope of the claim, the amount of direction or guidance provided, the lack of sufficient working

examples, the unpredictability in the art and the amount of experimentation required to enable one of skill in the art to practice the claimed invention. The specification disclosure is insufficient to enable one skilled in the art to practice the invention as broadly claimed without an undue amount of experimentation.

The specification discloses only a fluorogenic or fluorescent reporter compound such as rhodamine 110 having the structure as shown in formula VII on page 17 linked to a caspase or an enzyme involved in the intracellular apoptosis cascade for the claimed method of identifying an immunosuppressive agent **inside** living cells (intracellular screening). The specification further discloses that cell free assays or assays using dead, permeabilized cells, cannot predict the ability of compounds to penetrate cellular membrane. Using cell-free or dead cell assays, it would be virtually impossible to identify cell-type or organ specific modulators of the caspase cascade. It is possible that a compound identified in a cell free or dead cell caspase assay will not work in living cells (see page 6 of specification).

The specification does not teach how to make *any* reporter compound for the claimed method as set forth in claims 1-13 and 28-31 because there is insufficient guidance as to the structure of any reporter compound, let alone having any measurable property which is responsive to which undisclosed caspase cascade. Given the indefinite number of reporter compound, it is unpredictable which undisclosed reporter compound or substrate is responsive for which caspase cascade inside live cells, in turn, would be useful for the claimed method.

Stryer *et al* teach that a protein is highly dependent on the overall structure of the protein itself and that the primary amino acid sequence determines the conformational of the protein (See enclosed appropriate pages).

For these reasons, it would require undue experimentation of one skilled in the art to practice the claimed invention. See page 1338, footnote 7 of Ex parte Aggarwal, 23 USPQ2d 1334 (PTO Bd. Pat App. & Inter. 1992).

In re wands, 858 F.2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988), the decision of the court indicates that the more unpredictable the area is, the more specific enablement is necessary. In view of the quantity of experimentation necessary, the limited working examples, the unpredictability of the art, the lack of sufficient guidance in the specification and the breadth of the claims, it would take an undue amount of experimentation for one skilled in the art to practice the claimed invention.

10. Claims 1-31 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

The specification does not reasonably provide a **written description** of *any* reporter compound having *any* one measurable property, which is responsive to any caspase cascade.

The specification discloses only a fluorogenic or fluorescent reporter compound such as rhodamine 110 having the structure as shown in formula VII on page 17 linked to a caspase or an enzyme involved in the intracellular apoptosis cascade for the claimed method of identifying an immunosuppressive agent **inside** living cells (intracellular screening). The specification further discloses that cell free assays or assays using dead, permeabilized cells, cannot predict the ability of compounds to penetrate cellular membrane. Using cell-free or dead cell assays, it would be virtually impossible to identify cell-type or organ specific modulators of the caspase cascade. It is possible that a compound identified in a cell free or dead cell caspase assay will not work in living cells (see page 6 of specification).

With the exception of the specific reporter compound rhodamine 110 linked to the specific caspase for the claimed method of identifying immunosuppressive agent inside living cells, there is insufficient written description about the structure associated with function of any reporter compound, any properties of any reporter compound that is responsive for any caspase cascade for the claimed method. Thus, Applicant was not in possession of the claimed genus. *See University of California v. Eli Lilly and Co. 43 USPQ2d 1398.*

Applicant is directed to the Revised Interim Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

11. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

12. Claims 1-13 and 28-31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The recitation of "measurable property which is responsive to the caspase cascade" in claims 1, 2, 28 and 31 is indefinite and ambiguous because it is not clear which reporter compound and which property of the undisclosed reporter compound is used to detect which caspase activity.

13. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office Action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

14. Claims 1-11, 14-25 and 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Evans *et al* (Cancer Research 54: 1596-1603, March 1994; PTO 892).

Evans *et al* teach a method for identifying an immunosuppressive agent such as cisplatin comprising the steps (a) obtaining a population of viable cultured active T cells such as proliferating rat thymocytes or resting T cells such as quiescent rat thymocytes having intact membranes from a cell growth medium such as PRMI 1640 under conditions conductive to growth such as incubated at 37°C, which is about 42°C, (b) incubating the reference active and resting T cells in the presence various test compound such as MP, etoposide or Cisplatin (CP) dissolved in a solvent such as dimethyl sulfoxide (DMSO) or vehicle (DMSO) alone over a predetermined period of time such as 12, 24, 36 or 48 hours (See page 1597, column 1, Materials and Methods, isolation of Immature Rat Thymocytes, Figure 1, in particular); (c) separately adding to each culture such as Cisplatin treated, etoposide treated and vehicle control a reporter compound such as TB or acridine orange (AO) which is responsive to the caspase cascade such as cell viability or apoptosis; (d) measuring at least one measurable property such as uptake of TB and the fluorescence of acridine orange (AO) under fluorescence microscopy (visualization) for nuclear condensation, cell morphology such as membrane intactness. Cells undergoing apoptosis which is associated with caspase cascade are readily distinguishable from the number of viable cells since the latter displayed diffuse nuclear staining patterns (See page 1597, Assessment of Cell Viability, in particular) and (e) calculating the caspase activity by tabulating the number of

nonviable and apoptotic cells for cisplatin treated cells (First volume) and the number of nonviable and apoptotic cells for vehicle (second volume). The reference test compound is applied to the T cells at a concentration such as 10-50μM) which is within about 1 picomolar to about 1 millimolar. The reference method further comprises adding a permeabilization enhancer such as triton X100 in PBS in combination with the reference reporter compound such as propidium iodide or permeabilized enhancer such as DMSO in combination with the reference reporter compound such as acridine orange supplied by Molecular Probes, Inc (See page 1597, column 2, Cell Cycle Analysis or Unfractionated and Purified Thymocyte Populations, Materials and Methods, in particular). The viable cultured cells in the reference method are in separate wells of a microplate such as 96-well immunoassay plates (See page 1598, column 1, first paragraph, in particular). The arbitrary ratio such as when the first ratio is greater than one as recited in claim 1 is within the teachings of Evans *et al* who teaches that following treatment with MP and ectoposite, both proliferating (active) and quiescent (resting) thymocytes exhibited a highly significant increases in the amount of apoptosis detected above control levels (See page 1600, column 2, first paragraph, in particular). Thus, the reference teachings anticipate the claimed invention.

15. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 103(a) that form the basis for the rejections under this section made in this Office Action:

A person shall be entitled to a patent unless:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. This application currently names joint inventors. In considering Patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

17. Claims 1, 12, 14 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Evans *et al* (Cancer Research 54: 1596-1603, March 1994; PTO 892) in view of Wesselborg *et al* (Eur J Immunol 23(10): 2707-10, Oct 1993; PTO 892) and Hug *et al* (Biochemistry 38: 13906-13911, 1999; PTO 1449).

The teachings of Evans *et al* have been discussed supra.

The claimed invention in claims 12 and 26 differs from the teachings of the reference only that the method wherein the active T cells are obtained by adding to T cells antibodies to the T cell receptor, Concanavalin A or Phytohaemagglutinin.

Wesselborg *et al* teach that anti-CD3 TcR monoclonal antibodies trigger apoptosis in activated but not resting mature peripheral T cells (See abstract, in particular).

Hug *et al* teach a method of detecting caspase activity in intact cells using reporter compound such as rhodamine 110 coupled to amino acids or peptides as substrates in any cells (See entire document, page 13906, column 2, second paragraph, in particular). Hug *et al* teach that it is difficult to detect apoptosis in vivo and ex vivo since the apoptotic cells are removed by cells with phagocytic activity and rhodamine 110 coupled to amino acids or peptides of caspase substrate is capable of detecting caspase in early apoptosis; the reference reporter compound is more sensitive than conventional PhiPhiLux staining and capable of penetrates the cell membrane (See page 13910, column 2, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to identifying any immunosuppressive agent using active T cell by adding T cells antibodies to the T cell receptor using as well as resting T cells as taught by Wesselborg *et al* using a reporter compound as rhodamine 110 coupled to caspase peptide or amino acid as taught by Hug *et al*. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Wesselborg *et al* teach that apoptosis is cell type specific and antibody such as anti-CD3TcR monoclonal antibodies trigger apoptosis only in activated but not resting mature peripheral T cells (See abstract, in particular). Hug *et al* teach that it is difficult to detect apoptosis in vivo and ex vivo since the apoptotic cells are removed by cells with phagocytic activity and rhodamine 110 coupled to amino acids or peptides are capable of detecting caspase in early apoptosis; the reference reporter compound is more sensitive than conventional PhiPhiLux staining and capable

of penetrates the cell membrane (See page 13910, column 2, in particular). Evans *et al* teach that cell undergoing apoptosis is associated with activation of caspase which can be readily distinguishable from the number of viable cells since the latter displayed diffuse nuclear staining patterns (See page 1597, Assessment of Cell Viability, in particular).

18. Claims 1, 2, 13-15, and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Evans *et al* (Cancer Research 54: 1596-1603, March 1994; PTO 892) in view of Zeher *et al* (Arthritis Rheum 43(5): 1187-8, May 2000; PTO 892) and Hug *et al* (Biochemistry 38: 13906-13911, 1999; PTO 1449).

The teachings of Evans *et al* have been discussed supra.

The claimed invention in claims 13 and 27 differs from the teachings of the reference only that the method wherein the active T cells are obtained from tissue of a patient afflicted with one or more immunopathological symptoms and wherein said resting T cells are from healthy tissue that is not afflicted with the immunopathological symptoms.

Zeher *et al* teach that activated T cells obtained from tissue of a patient afflicted with immunopathological symptoms such as primary Sjogren's syndrome are susceptible to apoptosis induced by various anti-CD3, anti-CD95 monoclonal antibodies and ionophore treatment. Zeher *et al* further teach that there is a positive correlation between the increased susceptibility to apoptosis of peripheral CD4+ T cells and activity of disease from patient with Sjogren's syndrome compared with resting T cells from healthy tissue that is not afflicted with the immunopathological symptoms (See abstract, in particular).

Hug *et al* teach a method of detecting caspase activity in intact cells using reporter compound such as rhodamine 110 coupled to amino acids or peptides as substrates in any cells (See entire document, page 13906, column 2, second paragraph, in particular). Hug *et al* teach that it is difficult to detect apoptosis in vivo and ex vivo since the apoptotic cells are removed by cells with phagocytic activity and rhodamine 110 coupled to amino acids or peptides are capable of detecting caspase in early apoptosis; the reference reporter compound is more sensitive than conventional PhiPhiLux staining and capable of penetrates the cell membrane (See page 13910, column 2, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use tissue from patient afflicted with any one or more immunopathological symptoms and resting T cells from healthy tissue as control as taught by Zeher *et al* for a method

of identifying immunosuppressive agent by detecting caspase activity and/or cell viability using reporter compound such as rhodamine 110 coupled to caspase peptide substrate as taught by Hug *et al* or acridine orange (AO) as taught by Even et al. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Zeher *et al* teach that there is a positive correlation between the increased susceptibility to apoptosis of peripheral CD4+ T cells and activity of disease from patient with Sjogren's syndrome compared with resting T cells from healthy tissue that is not afflicted with the immunopathological symptoms (See abstract, in particular). Hug *et al* teach that it is difficult to detect apoptosis in vivo and ex vivo since the apoptotic cells are removed by cells with phagocytic activity and rhodamine 110 coupled to amino acids or peptides are capable of detecting caspase in early apoptosis; the reference reporter compound is more sensitive than conventional PhiPhiLux staining and capable of penetrates the cell membrane (See page 13910, column 2, in particular). Evans *et al* teach that cell undergoing apoptosis is associated with activation of caspase which can be readily distinguishable from the number of viable cells since the latter displayed diffuse nuclear staining patterns (See page 1597, Assessment of Cell Viability, in particular).

19. Claims 28-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Evans *et al* (Cancer Research 54: 1596-1603, March 1994; PTO 892) in view of Migita *et al* (Transplantation 64(9): 1365-9, Nov 1997; PTO 892) and Hug et al (Biochemistry 38: 13906-13911, 1999; PTO 1449).

The teachings of Evans *et al* have been discussed supra. Evans et al further teach plurality of populations of viable cultured active T cells are in separate wells of a microtiter plate such as 96-well immunoassay plates (See page 1598, column 1, first paragraph, in particular).

The claimed invention in claim 28 differs from the teachings of the reference only that the method comprises exposing a first portion of at least one population to a combination of a predetermined amount of test compound and a subinducing amount of known immunosuppressant for a first predetermined period of time at a predetermined temperature.

The claimed invention in claim 29 differs from the teachings of the reference only that the method wherein a plurality of populations of viable cultured active T cells are exposed separately to a plurality of test compounds.

The claimed invention in claim 30 differs from the teachings of the reference only that the method wherein the plurality of populations of viable cultured active T cells are in separate wells of a microtiter plate.

Migita *et al* teach that immunosuppressant compounds such as FK506 and glucocorticoids are used for allograft rejection, graft-versus-host disease and autoimmune diseases. Thymocytes and mature human peripheral blood T cells undergo apoptosis by exposure to compound such as dexamethasome in vitro. Migita *et al* further teach that FK506, which is one of the immunosuppressive compound for allograft rejection, graft-versus host disease and autoimmune disease, synergistically enhanced dexamethasone mediated apoptosis which is associated with activation of caspase cascade in human peripheral blood T cells by exposing T cell to a combination of test compounds such as dexamethasome and a subinducing amount of known immunosupprersant such as FK506 (See abstract, in particular).

Hug *et al* teach a method of detecting caspase activity in intact cells using reporter compound such as rhodamine 110 coupled to amino acids or peptides as substrates in any cells (See entire document, page 13906, column 2, second paragraph, in particular). Hug *et al* teach that it is difficult to detect apoptosis in vivo and ex vivo since the apoptotic cells are removed by cells with phagocytic activity and rhodamine 110 coupled to amino acids or peptides are capable of detecting caspase in early apoptosis; the reference reporter compound is more sensitive than conventional PhiPhiLux staining and capable of penetrates the cell membrane (See page 13910, column 2, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to assay the potency of a test compound to synergize with any known immunosuppressive agent by exposing a portion of activated T cells to a combination of predetermined amount of test compound and a subinducing amount of immunosuppressant for a predetermined amount of time as taught by Migita *et al* by detecting caspase activity and/or cell viability using reporter compound such as rhodamine 110 coupled to caspase peptide substrate as taught by Hug *et al* or acridine orange (AO) as taught by Even *et al* using 96-wells microtiter plate. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Migita *et al* teach that exposing activated T cells to a combination of known immunosuppressive compounds can determine the synergistic effects of said compounds (See abstract, in particular).

Hug *et al* teach that it is difficult to detect apoptosis in vivo and ex vivo since the apoptotic cells are removed by cells with phagocytic activity. However, rhodamine 110 coupled to caspase amino acids or peptides substrate is capable of detecting caspase in early apoptosis; the reference reporter compound is more sensitive than conventional PhiPhiLux staining and capable of penetrates the cell membrane (See page 13910, column 2, in particular). Evans *et al* teach that cell undergoing apoptosis is associated with activation of caspase which can be readily distinguishable from the number of viable cells since the latter displayed diffuse nuclear staining patterns (See page 1597, Assessment of Cell Viability, in particular). Evans et al further teach that plurality of populations of viable cultured active T cells can be culture in separate wells of a microtiter plate such as 96-wells immunoassay plates (See page 1598, column 1, first paragraph, in particular).

20. Claims 14-17 and 22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Evans *et al* (Cancer Research 54: 1596-1603, March 1994; PTO 892) in view of Lesage *et al* (J Immunol 159(10): 4762-71, Nov 1997; PTO 892) or Bradbury *et al* (J Immunol Methods 240(1-2): 79-92, June 2000; PTO 892).

The teachings of Evans *et al* have been discussed supra.

The claimed invention in claims 16 and 22 differs from the teachings of the reference only that the method further comprises assessing cell viability by observing mitochondrial activity.

Lesage *et al* teach a method of assessing cell viability and sensitivity to apoptosis by observing mitochondrial activity such as a reduction in mitochodrial membrane potential (delta psi(m)) or production of reactive oxygen species in immature and mature T cells after induced by apoptotic stimuli (See abstract, in particular). Lesage *et al* teach that alternative apoptotic pathway may be involved in the susceptibility of cell death in T cells and cell death is characterized by a reduction in mitochondrial membrane potential, production of reactive oxygen species, loss in membrane asymmetry, exposure of phosphatidylserine residues and incorporation of vital dyes which is not block by treatment with caspase inhibitor (See abstract, in particular).

Bradbury *et al* teach that apoptosis is accompanied by opening of the mitochondrial transmembrane potential (DeltaPsi (m)) and caspase activation due the release of cytochrome c that could be measured by assessing fluorescent cyanine dye JC-1 (reporter compound) and the loss of functioning of mitochondrial transmembrane potential results in red fluorescence (See

abstract, in particular). Bradbury *et al* teach that the ADP:ATP ratio (mitochondrial transmembrane potential) is significantly correlated with the degree of apoptosis measured by TUNEL, and hypodipolidy measured by propidium iodide (See abstract, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to measure mitochondrial activity as taught by Lesage *et al* or Bradbury *et al* for a method of identifying any immunosuppressive agent as taught by Evans *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Bradbury *et al* teach that the ADP:ATP ratio (mitochondrial transmembrane potential) is significantly correlated with the degree of apoptosis measured by TUNEL, and hypodipolidy measured by propidium iodide (See abstract, in particular). Lesage *et al* teach that alternative apoptotic pathway may be involved in the susceptibility of cell death in T cells and cell death is characterized by a reduction in mitochondrial membrane potential, production of reactive oxygen species, loss in membrane asymmetry, exposure of phosphatidylserine residues and incorporation of vital dyes which is not block by treatment with caspase inhibitor (See abstract, in particular).

21. No claim is allowed.

22. Any inquiry concerning this communication or earlier communications from the examiner should be directed to "Neon" Phuong Huynh whose telephone number is (703) 308-4844. The examiner can normally be reached Monday through Friday from 9:00 am to 6:00 p.m. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (703) 308-3973. Any inquiry of a general nature or relating to the status of this application should be directed to the Technology Center 1600 receptionist (customer service) whose telephone number is (703) 872-9305.

23. Papers related to this application may be submitted to Technology Center 1600 by facsimile transmission. Papers should be faxed to Technology Center 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center telephone number is (703) 305-7401. The IFW official Fax number is (703) 872-9306. For After Final, the Fax number is (703) 872-9307.

Phuong N. Huynh, Ph.D.
Patent Examiner
Technology Center 1600
August 11, 2003

CHRISTINA CHAN
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600